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Oxygen Consumption of Tilapia and Preliminary Mass Flows through a Prototype Closed Aquaculture System

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TABLE OF CONTENTS

Table of contents.....	ii
List of figures.....	iii
List of tables.....	iv
Abstract.....	1
Introduction.....	1
Methods and Materials.....	2
Results and Discussion.....	6
CAS Performance.....	6
Oxygen Consumption.....	7
Carbon Dioxide Production and Respiration Quotients.....	8
Nitrogen Balance.....	13
Carbon Balance.....	16
Conclusion.....	17
Literature Cited.....	18

LIST OF FIGURES

- FIGURE 1. A prototype Closed Aquaculture System (CAS)
- FIGURE 2. Total CO₂ and O₂ quantification methods
- FIGURE 3. Total O₂ consumption averages preceding and including Trial 1
- FIGURE 4. Total moles of O₂ and CO₂ versus time for one cycle
- FIGURE 5. Consumption/production rates versus time for one cycle
- FIGURE 6. Microbial autotrophic oxygen demand
- FIGURE 7. Nitrogen inputs and resevoirs which must be quantified for mass closure
- FIGURE 8. The percentage of nitrogen closure for five respiration trials
- FIGURE 9. Preliminary carbon resevoirs for one respiration trial

LIST OF TABLES

- TABLE 1. Fish quantities and length of data collection for five respiration study trials
- TABLE 2. Typical headspace O₂/CO₂ trends for a series of cycles
- TABLE 3. Oxygen consumption averages for five respiration trials
- TABLE 4. Growth indices for five respiration trials
- TABLE 5. Protein indices for five respiration trials

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Abstract

Performance of NASA's prototype CELSS Breadboard Project Closed Aquaculture System was evaluated by estimating gas exchange quantification and preliminary carbon and nitrogen balances. The total system oxygen consumption rate was $535 \text{ mg hr}^{-1} \text{ kg-fish}^{-1}$ (cv=30%) when stocked with Tilapia aurea populations (fresh weights of 97 ± 19 to $147 \pm 36 \text{ g fish}^{-1}$ for various trials). During the artificially lighted day (10 hrs, approx. $20 \text{ umol m}^{-2} \text{ s}^{-1}$, incandescent), total oxygen consumption averaged $590 \text{ mg hr}^{-1} \text{ kg fish}^{-1}$ (cv=28%), while dark consumption measured $470 \text{ mg hr}^{-1} \text{ kg-fish}^{-1}$ (cv=29%). Oxygen consumption by T. aurea ($260 \text{ mg hr}^{-1} \text{ kg-fish}^{-1}$) contributed to approximately one-half of total system demand. Continuous carbon dioxide quantification methods were analyzed using the relation of carbon dioxide production to oxygen consumption. Overall food conversion rates averaged $18.2 \pm 3.2\%$. Net protein retention averaged $21.3 \pm 3.7\%$. Major pathways for nitrogen and carbon in the system were described with preliminary mass closures of 60-80% and 60% for nitrogen and carbon.

Introduction

Mass flow quantification through all potential components of a bioregenerative life support system is a central goal for NASA's Controlled Ecological Life Support System (CELSS) research program. An expanding aspect of this effort focuses on biological systems designed to recover resources present in CELSS waste streams, including inedible plant matter from the major food production component and human urine, feces and hygiene water.

One proposed resource recovery scenario includes the use of aquaculture to recycle portions of these streams and produce a protein-rich secondary food source for human consumption (Muller, in process). As previous CELSS research has focused on mass flows of gases through a closed plant

growing system (Wheeler and Sager, 1990), oxygen and carbon dioxide exchange rates for a closed aquaculture system are of primary interest. Additionally, the possible combination of aquaculture components with plant nutrient and other Resource Recovery systems requires the monitoring of pertinent water chemistry parameters and recognition of potential carbon and nitrogen sinks. The popular aquaculture species Tilapia was chosen for initial CELSS investigations because they are generally omnivorous, rapid growers, easy to reproduce and adjust well to closed, intensive culture (Stickney, 1986; Rothbard et al., 1975). This study targeted evaluation of a prototype Closed Aquaculture System (CAS) hardware and software design and initial quantification of mass flows.

The CAS was monitored for oxygen depletion and carbon dioxide production to determine total system respiration. The ability to monitor actual respiration of a system depends on accurate quantification of O₂ depletion and CO₂ production. While O₂ depletion is readily measurable, quantification of dissolved CO₂ has proved difficult (Weatherly, 1972; Brett and Groves, 1979). The most significant factors affecting calculation methods applied in this study were the small headspace to water volume ratio and equilibrium pH. In the CAS, approximately 75% of the CO₂ produced by fish and microbe respiration was partitioned in the water.

Interval removal of Tilapia from the system allowed approximation of fish respiration. Water quality measurements included organic and inorganic carbon and nitrogen as well as various plant nutrients. Tilapia growth and feed conversion efficiencies were determined.

Materials and Methods

Tilapia of similar size (fresh weights of 97 \pm 19 to 147 \pm 36 g fish⁻¹ for various trials) were stocked into the prototype CAS for routine activity respiration study. The

atmospherically and hydrologically closed system consisted of a 460 L fish tank, fixed-film biofilter, downflow solids-retention sand filters, heater, pump and associated hardware (Figure 1).

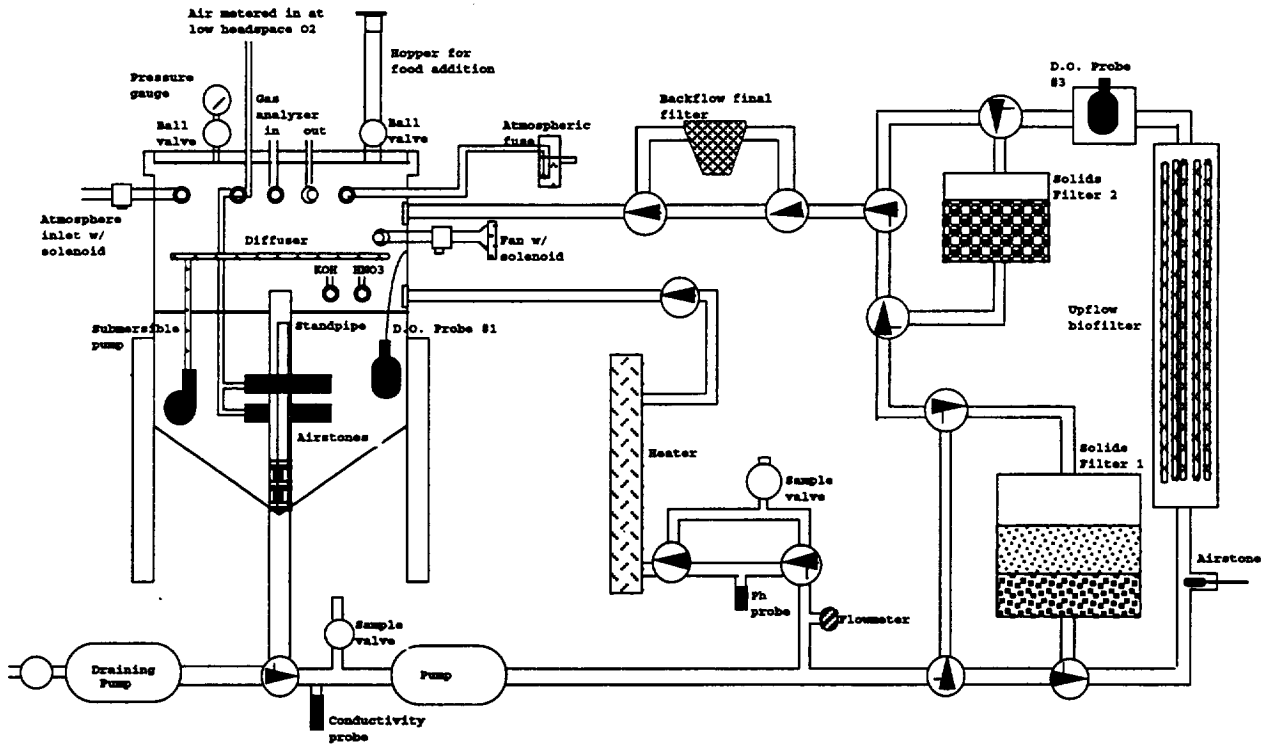


Figure 1. The prototype Closed Aquaculture System (CAS).

Automated control of sealed status and system monitoring was performed by a desktop host computer, with data recorded at five minute intervals. On-line monitoring parameters included temperature, pH, conductivity, headspace carbon dioxide (CO₂) and oxygen (O₂), dissolved O₂, flow rates and valve status. Water samples were collected daily and assayed for carbon and nitrogen forms.

Removal and stocking of fish was performed over several cycles to determine respiration of the system with and without fish (to isolate associated microbial biomass effects). Individual cycles represented the period of time for which the system was atmospherically sealed. Sealed status was automatically terminated upon dissolved oxygen

levels reaching 4.8 mg L⁻¹. Atmospheric air was bubbled through the system to replenish O₂ prior to the initiation of the next cycle. Cycles lasted approximately 4 to 12 hours, depending on quantity of fish stocked, activity and the extent of microbial growth.

The system respiration rate was estimated using linear regression analysis of total O₂ and CO₂ over time. Total O₂ calculations were based on measured headspace O₂ partial pressure and dissolved O₂. Total CO₂ quantification utilized only headspace CO₂ partial pressure measurements, with continuous calculation of dissolved forms using Henry's Law for dissolved CO₂ and ionization constants for the bicarbonate and carbonate forms (Figure 2) (Stumm and Morgan, 1981).

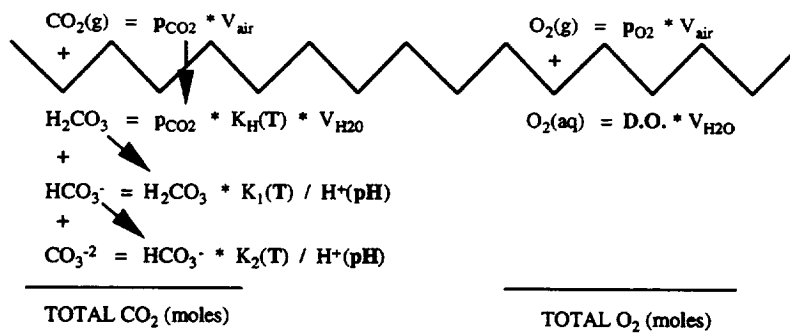


Figure 2. Total CO₂ and O₂ quantification methods. Measured parameters (bold print) were utilized to continuously calculate total quantities. Rate calculations based on changes in total.

Calculations were automated and tabulated every five minutes based on instantaneous headspace CO₂, pH and temperature. O₂ and CO₂ production rates were computed on changes in these total quantities.

System respiration rates were divided into light and dark periods to determine diurnal effects. The first three hours of data from the dark period were excluded from these calculations. Fish received 10 hours of standard incandescent room light a day (approx. 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Quantification of fish behavioral activity was not performed for this study.

The rate of fish O₂ consumption was calculated from the difference in O₂ depletion rates immediately preceding and following a fish stocking. It was determined the fish removal process modified the system environment to an extent which invalidated respiration quantification immediately after a fish removal. Biofilter instability during the initial phases of this study (sloughing of visually significant amounts of microbial biomass into the tank) affected system respiration. Significant efforts were made to reduce this impact including the addition of solids filters preceding and following the biofilter.

Several water changes during the study were performed, separating respiration cycles into five trials. Trial length, fish number, average and total initial weights stocked are shown (Table 1). Water volume in Trial 4 was reduced to correlate system loading.

Table 1. Fish quantities and length of data collection for five respiration study trials.

Trial	Fish No.	Trial Length, d	Avg. Wt. (+/-), g	Total Wt, g	Stocking Density, kg/m ³
1	20	12	97 (19)	1940	6.5
2	16	20	147 (36)	2105	7.0
3	17	33	134 (31)	2282	7.6
4	5	23	145 (8)	728	6.0
5	15	28	144 (16)	2160	7.2

Mass balances were approximated for several trials through examination of system inputs and outputs, including proximate analysis of fish food and fish tissue, analysis of daily water samples and removed biofilter solids, and average respiration rates. For this study, proximate analysis data was taken from subjects of a previous study (Owens and Hall, 1990) where *Tilapia aurea* were fed the identical food source (Purina Trout Chow, 43% protein, 3760 kcal kg⁻¹).

Throughout the study, water temperature was maintained between 25°C and 28°C and equilibrium pH between 6.6 and 6.8. Due to large build-ups of CO₂, short-term reductions in pH

were corrected by the automated pH controller with addition of 1 M KOH. Likewise, at valve or system openings, release of CO₂ and decline in associated carbonic acid resulted in short-term pH increases corrected by the addition of 1 M H₂SO₄. Sulfuric acid replaced nitric acid used in trial 1 to reduce denitrification potential and obscured nitrogen partitioning.

Results and Discussion

CAS Performance. Evaluation of the prototype CAS as a tool for accurate quantification of CELSS aquaculture mass flows was performed. Upon determination of negligible atmospheric leakage (Dreschel et al., 1991), water and fish were added to initiate hardware and software assessment. Data collection and control software performed to specifications. Several modifications to hardware configuration were necessary because of observed system instabilities. Uncontrolled transfer and suspension of microbial biomass from the biofilter to the fish tank was corrected with the addition of solids filters. CO₂ build-up across individual cycles was only partially corrected with the addition of a spray ring and additional air flow through tank headspace to increase mass transfer across the gas-liquid interface. Cyclical O₂/CO₂ headspace concentrations for several cycles is shown (Figure 2).

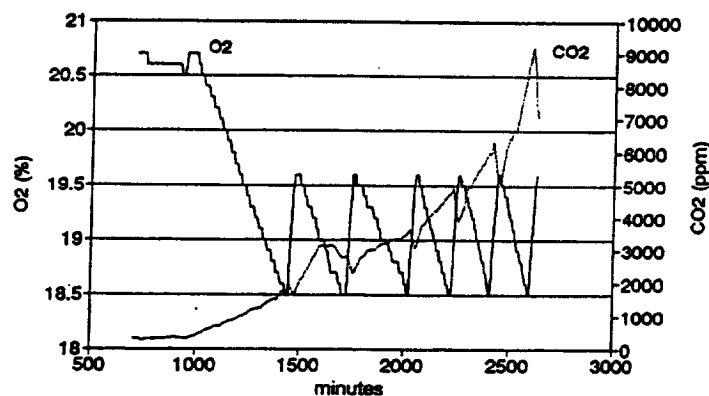


Figure 2. Typical headspace O₂/CO₂ trends for a series of cycles.

Biofilter performance was nominal, with temporary increases in $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ reduced through autotrophic oxidation to $\text{NO}_3\text{-N}$.

Oxygen Consumption. O_2 consumption averages for each trial are summarized (Table 1), with an example of consumption rates of individual cycles preceding and including Trial 1 shown (Figure 3). Linear regression analysis of individual cycles consistently showed high linear conformity, with $R^2 > 0.95$. O_2 consumption rates after water changes gradually increase, reflecting increasing microbial load and to a lesser extent, fish growth. Average system consumption for all trials was $535 \text{ mg hr}^{-1} \text{ kg fish}$ (cv=30%). O_2 consumption with lights on averaged $590 \text{ mg hr}^{-1} \text{ kg fish}$ (cv=28%) and $470 \text{ mg hr}^{-1} \text{ kg fish}$ (cv=29%) with lights off.

O_2 consumption by *T. aurea* was responsible for about one-half of the total system O_2 use. Differences before and after fish stocking averaged $260 \text{ mg hr}^{-1} \text{ kg fish}$ (cv=28%), a value concurring with routine activity rates reported in previous metabolic research on tropical fish species (Brett and Groves, 1979; Weatherly and Gill, 1987).

Table 3. Oxygen consumption averages ($\text{mg hr}^{-1} \text{ kg fish}^{-1}$) for five respiration trials.

Trial	Total System (cv%)	Lights On (cv%)	Lights Off (cv%)
1	510 (30%)	580 (30%)	460 (29%)
2	530 (35%)	585 (30%)	490 (30%)
3	565 (25%)	605 (25%)	525 (25%)
4	445 (18%)	515 (20%)	380 (28%)
5	580 (33%)	640 (30%)	540 (30%)
TOTAL	535 (30%)	590 (28%)	470 (29%)

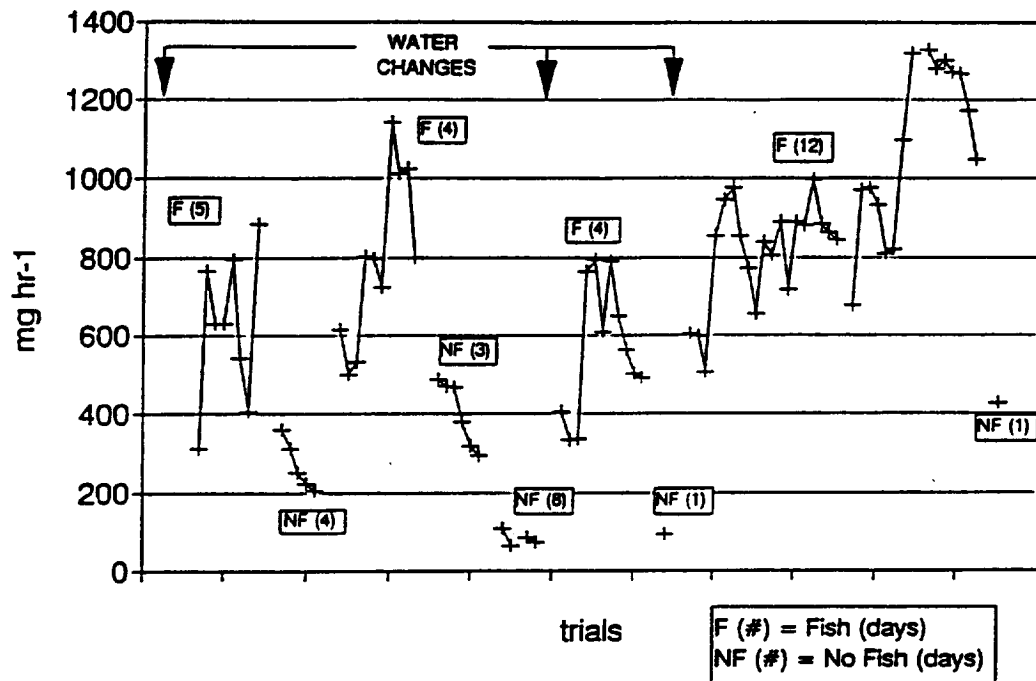


Figure 3. Total O₂ consumption averages preceding and including Trial 1. Each point represents an average system consumption for one cycle.

Rates of oxygen consumption by suspended microbes without fish or attached biofilms were estimated from dissolved O₂ changes in water samples removed from the system during several trials. O₂ demand in these samples showed microbial demand split approximately in half for suspended and attached biofilms (primarily in biofilter). However, this ratio varied greatly with biofilter stability.

As anticipated, O₂ requirements by fish will not pose a significant demand on the gas exchange function of a CELSS plant growing system. Calculations show that one kilogram of fish consume the oxygen produced by approximately 0.15 m² of wheat being grown in NASA KSC's Biomass Production Chamber (Wheeler and Sager, 1990).

CO₂ Production and Respiration Quotients. The equilibrium dependence of solubility and dissociation constants used in the CO₂ calculations appears to hinder accurate on-line calculation using methods applied in this study. Computation of CO₂ production rates over an entire cycle often produced variable data with non-linear trends. The relationship of O₂ consumed to CO₂ produced was used to evaluate this variability. Respiration quotients (RQ = CO₂ produced : O₂ consumed) have been used extensively in the study of metabolic energetics of homeotherms, having particular usefulness in providing information on the composition of substrates being metabolized (Kleiber, 1961; Guyton, 1990). Fish RQ should vary between 0.7 and 1.1 depending on the substrate being oxidized (Weatherley and Gill, 1987).

Programs were developed to automatically display current gas exchange trends to assist with the evaluation of CO₂ computation methods. The initial graphical output displays total CO₂ and O₂ (in moles) versus time. The trends from one cycle are displayed (Figure 4). A "rolling", twelve data point (the past one hour) regression of each line is performed every five minutes which displays near real-time consumption and production rates in a second graphical output. O₂ consumption is displayed as a positive value to allow direct comparison with CO₂ production (Figure 5).

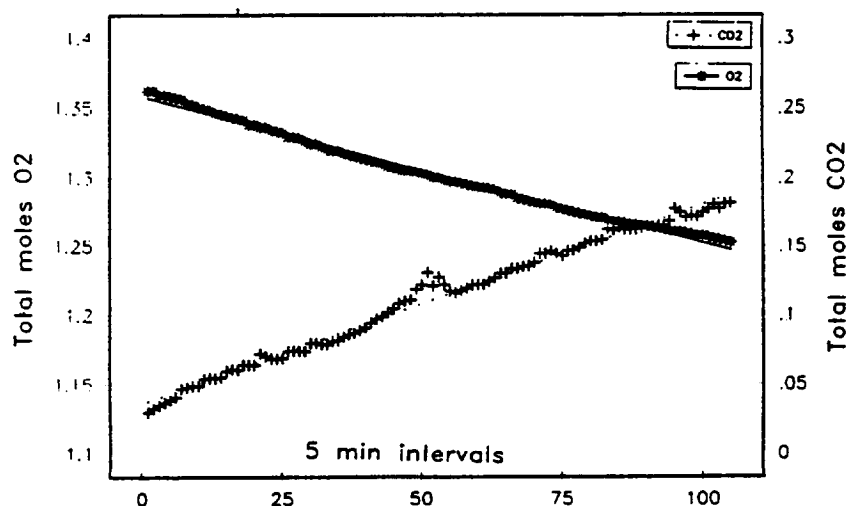


Figure 4. Total moles of O₂ and CO₂ versus time for one cycle.

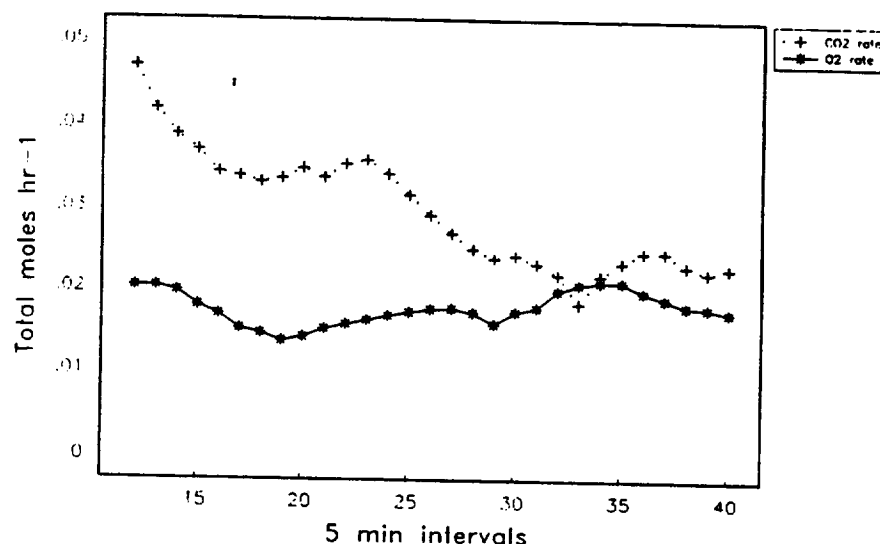


Figure 5. Consumption/production rates versus time for one cycle. Calculated with a "rolling", one-hour regression of total moles present. O₂ demand is shown as a positive value to allow for direct comparison with CO₂ production.

The trend displayed represents one typical cycle during a trial and highlights the calculation's limitations. The calculation greatly overestimates CO₂ production rates during the first 2-3 hours of the cycle. This is caused by greater amounts of dissolved CO₂ present than would be predicted by headspace partial pressure and the solubility constant. When the system is unsealed at the termination of the preceding cycle, headspace CO₂ levels rapidly equilibrate with surrounding atmospheric concentrations ($\Delta 10,000\text{ppm}$). However, as the transfer of CO₂ from aqueous to gas phase is a relatively slow process (compared to ionization rates), the water does not reach equilibrium with this headspace partial pressure in a timely fashion. As headspace partial pressure is the only inorganic carbon measurement taken, the calculation underestimates total CO₂ present. Once the system is re-sealed, the gas-liquid interface begins to approach equilibrium by continuing re-distribution of CO₂ into the headspace, which now rapidly increases in partial pressure. This rate is interpreted as system CO₂ production by the calculation, not a re-distribution, because pH is held

relatively constant with automated pH control. Normally, a pH increase is expected with re-distribution of CO₂ from liquid to gas phase. As the system approaches equilibrium across the interface, the "production" rate falls to equate closely with the O₂ consumption rate.

In summary, the data trends appear to be caused by insufficient purging between trials resulting in a non-equilibrium "surplus" of CO₂ in the water when the following cycle is initiated. Unfortunately, even with system improvements, the purging process is fairly inefficient and requires extremely long periods of time in between cycles to reach an approximate equilibrium. Compounding the problem, cycle length is dictated by adequate oxygen levels for the fish, so cycle length cannot be significantly extended to increase data collection after the period of re-distribution.

A limited RQ investigation for this study avoided non-equilibrium effects of CO₂ off-gassing from the water. RQ values were calculated after the system had been unsealed for an extended period, and the first 90 minutes of each cycle were excluded. The average RQ for ten cycles was 0.68 (cv=12.05). RQ of the system without fish (microbial) was tabulated on day 5, 10, 17 (day 0 = water change), and dropped from 0.80 to 0.69 to 0.42, respectively. A reduction of RQ with time was expected as the autotrophic microbial community grows, increasing the demand for O₂ without contributing to CO₂ production (oxidization of ammonia nitrogen to nitrate). RQ values for the fish were determined by

$$\text{fish RQ} = \frac{\text{total system CO}_2 \text{ produced} - \text{microbial CO}_2 \text{ produced}}{\text{total system O}_2 \text{ consumed} - \text{microbial O}_2 \text{ consumed}}$$

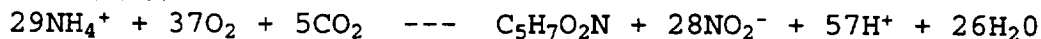
and averaged 0.75 (cv=5.76) for the three stockings performed after known equilibrium. This data appears to highlight a

potential application of RQ analysis in closed aquaculture systems.

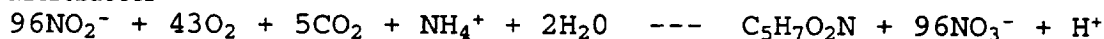
Automated RQ analysis could be used as a bioenergetic system parameter if accurate methods can be developed to allow continuous quantification of total CO₂. It is unlikely continuous RQ analysis could be used in aquatic systems to evaluate specific metabolic substrates of the fish alone. The respiration of microbial communities and gas-liquid phase interactions hopelessly obscure fish contributions. However, the system RQ may be of interest. Stoichiometric O₂ demand for an autotrophic community oxidizing ammonia excreted by the fish is approximately 65% of the average Tilapia O₂ demand in this study (Figure 6).

Microbial Autotrophic Oxygen Demand

Nitrosomonas



Nitrobacter



Fish Demand (1 kg Fish): Associated Autotrophic Microbial Demand*
6.2 g O₂ day⁻¹: 3.8 g O₂ day⁻¹

Figure 6. Microbial autotrophic oxygen demand based on 4g O₂ per 1g NH₄⁺ oxidized to NO₃⁻ (Wheaton, 1990), 0.033g NH₄⁺ excreted per 1g feed intake by fish (Colt and Orwicz, 1991), 3% feeding rate, fish O₂ consumption as reported in this study.

This demand forces a stabilized, closed aquaculture system RQ to vary between 0.55 and 0.65, depending on heterotrophic microbial demand in a specific system (a function of uneaten food quantities, biofilm surface area, etc). Variations in this system value could potentially provide information on a reduction in autotrophic microbial activity and the presence of any photoautotrophic activity during light cycles.

Growth. Fish growth was monitored to calculate feed conversion efficiencies, compute mass balances and present gas exchange data relative to the total amount of fish

present. Average growth indices are defined and reported for each trial (Table 4):

Table 4. Growth indices for five respiration trials.

$$\text{Specific Growth Rate (SGR)} = \frac{[\ln(\text{final wt}) - \ln(\text{initial wt})]}{\text{days}} \times 100$$

$$\text{Food Conversion Rate} = \frac{\text{weight gain (dry)}}{\text{food given (dry)}} \times 100$$

Trial	SGR	FCE
1	1.33	21.56
2	0.76	18.41
3	0.64	13.08
4	1.03	18.04
5	1.18	19.80

These growth rates are slightly lower than previous reports where Tilapia were grown on a similar food source (Winfree and Stickney, 1981; Shiau and Huang, 1989; Anderson et al., 1984). However, this is not unexpected with the frequent fish stocking and removal in these trials. Future studies should target greater system stabilities and extend trial length. However, these growth data are baseline targets for comparisons with alternative CELSS food sources.

Nitrogen Balance. Nitrogen inputs and reservoirs are presented (Figure 7). When attempting to balance nitrogen inputs with potential reservoirs, each reservoir must be quantified. When inspecting the various pathways between inputs and reservoirs in trial 1, a significant nitrate deficit was noticed. Nitrate accumulation in the CAS was expected because of both autotrophic conversion of ammonia (NH_4 to NO_2 to NO_3) and pH control addition of HNO_3 .

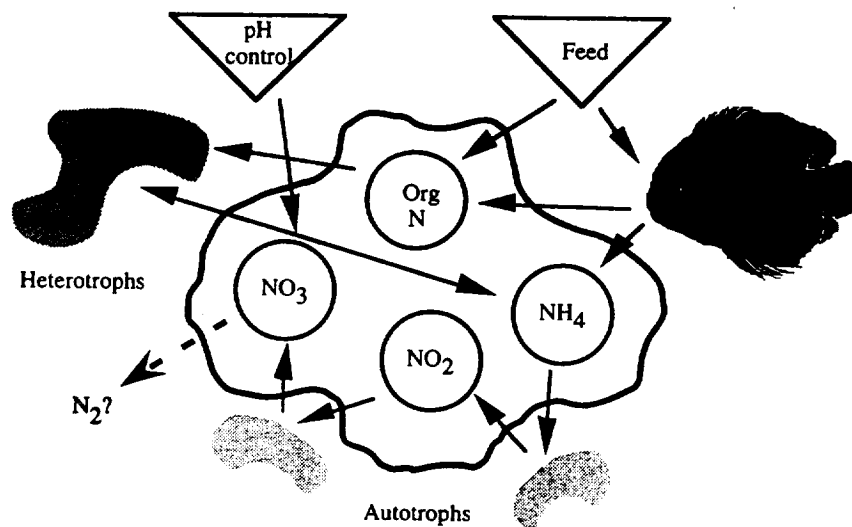


Figure 7. Nitrogen inputs and reservoirs which must be quantified for mass closure.

While some denitrification activity was possible, dissolved oxygen levels were typically above 4.5 mg L⁻¹ and make it an unlikely explanation for the entire missing quantity. Nitric acid was discontinued as a pH control solution after trial 1. The removal of this nitrogen input improved nitrogen budgeting efforts. The potential combination of aquaculture media and plant nutrient solutions make this an ideal acid in the future. However, in this study, attempts to track N partitioning appeared to be unnecessarily obscured.

The total percentage of nitrogen (reservoirs/inputs) accounted for each trial is shown (Figure 8). Fish tissue reservoirs were estimated from linearized total growth and prior tissue analyses. Soluble organic and inorganic nitrogen concentrations were multiplied by water volume to provide total quantity. While microbial content of nitrogen was minimal compared to fish biomass, it was estimated from microbial oxygen consumption data and previously reported overall cell composition (Niedhart et al. 1990).

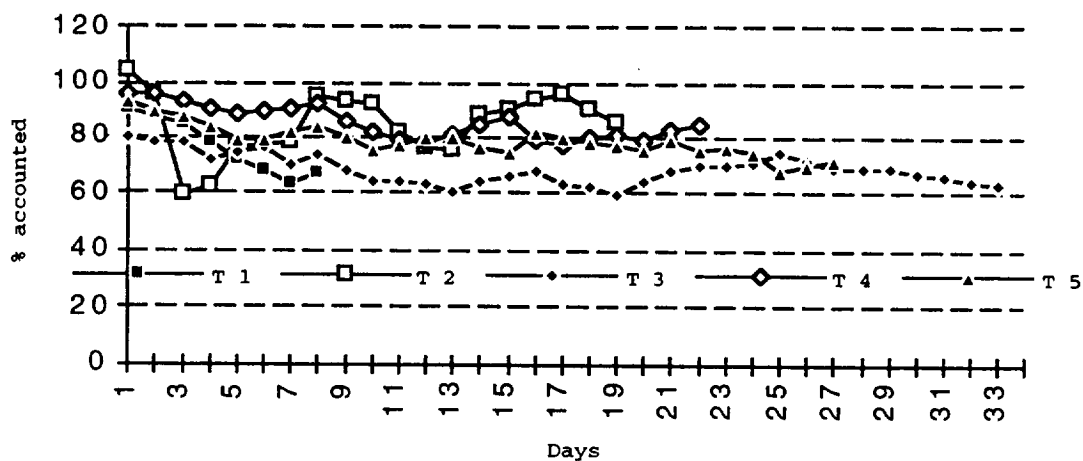


Figure 8. The percentage of nitrogen closure for five respiration trials.

Assuming fish tissue compositions in this study are similar to those of the previously mentioned control subjects, protein assimilation indexes are defined and shown for the five trials (Table 5).

Table 5. Protein indices for five respiration trials.

$$\begin{aligned} \text{Protein Efficiency Ratio (PER)} &= \frac{\text{live weight gain}}{\text{protein given}} \\ \text{Net Protein Retention (NPR)} &= \frac{\text{gain in tissue protein}}{\text{protein fed}} \times 100 \end{aligned}$$

Trial	PER	NPR
1	1.53	25.31
2	1.31	21.61
3	0.93	15.35
4	1.28	21.18
5	1.40	23.24

These indices will be contrasted with the alternative protein levels and protein : energy ratios resulting from diets derived from CELSS wastes. Previous research on T. aurea has reported higher protein assimilation efficiencies when

utilizing protein levels lower than those in the Phase I food source (Winfrey and Stickney, 1981; Davis and Stickney, 1978).

Carbon Balance. To model carbon flows through the CAS, elemental carbon analysis needs to be performed on fish tissue, feed and, possibly, biofilm. However, a preliminary balance was constructed at the end of Trial 1 that reflects inputs and reservoirs relevant to CELSS planning (Figure 9). The median system RQ value was used to determine CO₂ production and previous proximate analysis of feed and fish tissue used to estimate carbon input and storage. Assumptions made for the balance include a uniform carbon content of 50% in protein and a microbial assimilation efficiency of 40%.

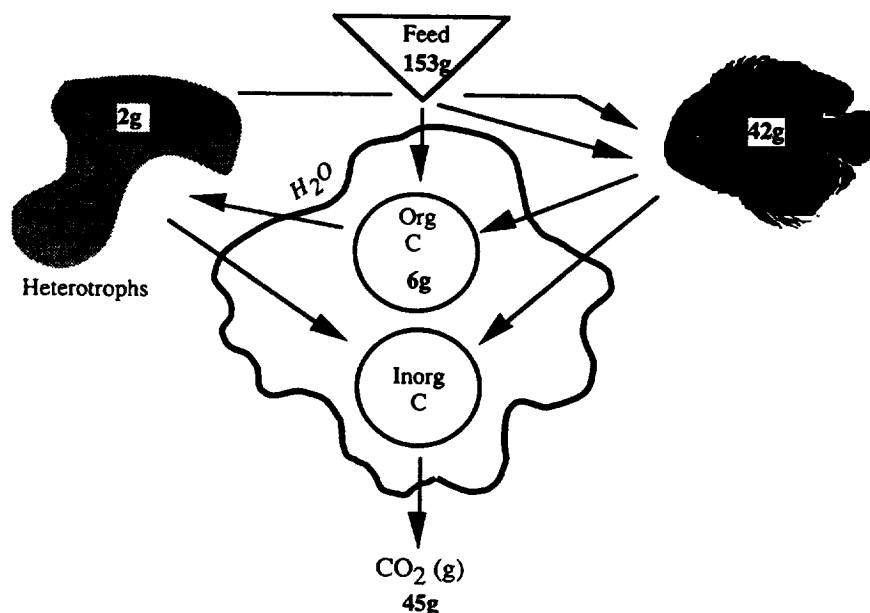


Figure 9. Preliminary carbon reservoirs for one respiration trial.

Based on this carbon balance, standard bioenergetic conversion coefficients were defined:

$$U-1 = \frac{\text{assimilated C}}{\text{consumed C}} = 51.1$$

$$K_1 = \frac{\text{wt. gain C}}{\text{consumed C}} = 27.6$$

$$K_2 = \frac{\text{wt. gain C}}{\text{wt. gain C} + \text{resp C}} = 53.9$$

Fischer (1979) documents energy conversion coefficients between 0.15 to 0.24 for Tilapia fed diets with varying degrees of algal, animal and plant components. Assimilation coefficients are reported as 0.50. As CELSS aquaculture research progresses and the feed stream shifts toward one of plant origin, these coefficients will decrease. The efficiency of energy conversion will remain a primary basis of appraising aquaculture as a method of secondary biomass production.

Conclusion

Oxygen demand by a Resource Recovery aquaculture component is readily measurable and will not impose a significant impact to gas exchange functions of a CELSS. Quantification of CO₂ production in the CAS is more difficult, but presents a unique opportunity to explore the use of respiration quotients as a bioenergetic aquaculture system parameter. An engineering evaluation of converting gas exchange quantification hardware from the current cyclical sealed-unsealed design to a continuous flow-through system should be accomplished.

Optimized fish growth should be targeted with system stabilization and extension of trial length. However, it is essential to quantify energy and protein conversion efficiencies with alternative CELSS diets to evaluate the incorporation of secondary fish production in a functioning CELSS.

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